Comparison of External RNA Controls on Different Technology Platforms

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Why do we want to compare different technologies with external RNA controls?

Objectives

- *Demonstrate application utilities of external RNA controls on different technology platforms, e.g. oligo vs. cDNA arrays, arrays vs. RT-PCR*
- *Provide end-users with useful references and guidelines for using the RNA controls in their own protocols*
- *Make it easier for end-users to compare experimental results cross different technologies*

Do we need to compare all existing technologies?

No, because

- ***Limited resources***
- ***Endless comparisons since everyone has his own favorite technology***

Basic Criteria of Comparability Test

- *Choose popular and representative technologies with a broad range of end-users*
- *Test with common and commercially available reagents and instruments, preferably from one source*
- *Establish simple and flexible protocols that can further expanded if necessary*

Technology Platforms

- *Microarray (s)*
- *RT-PCR (s)*
- *Others?*

Microarray Technology Platforms

- ***Affymetrix Gene-Chip®***
- ***Printed oligonucleotide microarray***
- ***cDNA microarray***
- ***Membrane-based macroarray***

Microarray Technology Platforms (Cont.)

- *One vs. Two-color labelling*
- *aRNA amplification or direct labeling*
- *Relative differential quantitation*
- *Pools, spiked pools, and encapsulated full process controls*

RT-PCR Technology Platform

- *One vs. Two enzymes*
- *Single vs. Two-tubes (cDNA vs. total RNA)*
- *Dye- vs. different probe-based*
- *Quantitation*
- *Pools, spiked pools, and encapsulated full process controls*

Basic Parameters for Comparability Test

- *Specificity*
- *Sensitivity and efficiency*
- *Linearity*
- *Quantitation ranges*
- *Precision and repeatability*
- *Detection and quantitation limits*

Discussion Points for Comparability

- ***Standard operation procedures***
- ***Inter-laboratory reproducibility***
- ***Data collection and acceptance criteria***
- ***Reference values***
- ***Statistical analysis***